AD

TECHNICAL REPORT

70-41-FL

THE MICROBIOLOGICAL WHOLESOMENESS OF SPACE FOODS

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June 1969

UNITED STATES ARMY
NATICK LABORATORIES
Natick, Massachusetts 01760

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FOREWORD

The microbiological requirements for space foods were established in 1964 to safeguard the health of the astronauts during a space mission. The scientific and technical rationale behind these requirements and the methodology for determining their compliance is presented. These requirements are being constantly reviewed and have been revised several times since they were first instituted, to reflect the contemporary state of knowledge. Revisions will continue to be made as experience and knowledge warrant it. National and international advice and collaboration to assess the feasability, effectiveness, and significance of these requirements and their respective analytical methods have always been sought and encouraged. A summary of the microbial analytical profile of random space food samples made at NLABs during 1967 and 1968 is presented. This work was done under Project No. 1J061102A71C, Food Research.

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ABSTRACT

The microbiological requirements for space foods were established in 1964 to safeguard the health of the astronauts during a space mission. These requirements are: the total aerobic plate count shall not exceed 10,000 per gram; the total coliform count shall not exceed 10 per gram; the fecal coliforms shall be negative in one gram; the fecal streptococci shall not exceed 20 per gram; the coagulase positive staphylococci shall be negative in five grams and the Salmonellae shall be negative in ten grams of food.

During 1967 and 1968, 88 percent of the space foods tested had total plate counts of less than 10,000 per gram; 96 percent had less than 10 coliforms per gram and 99 percent were negative for fecal coliforms; 86 percent had less than 20 streptococci per gram; 100 percent were negative for staphylococci: and 97 percent were negative for Salmonellae.

This report discusses the scientific and technical rationale behind these microbiological requirements. These requirements and the methodology prescribed for determining compliance are documented. Both are under continued review and amendment in keeping with up-to-date scientific knowledge and technical experience.

A. INTRODUCTION

About five years ago The U.S. Army Natick Laboratories provided the initial version of the currently instituted microbiological requirements and respective analytical methodology for space food prototypes. Four years ago the subject was discussed before an audience of industrial, academic and government (DOD and NASA) representatives assembled at the 1965 Spring meeting of the Research and Development Associates at Denver, Colorado*. Since that time, these requirements and methods have undergone several critical reviews, and five amendments have already been issued (Appendix) and a sixth amendment is in progress. Such reviews followed by appropriate readjustments shall always be undertaken as often as warranted by new scientific evidence or new experience.

It is acknowledged that these microbiological requirements and analytical methods are rather empirical in nature, and somewhat arbitrary but they are based upon the current state of the art, years of experience with these and related products, and the best scientific judgment. Specific analytical and clinical evidence are still urgently needed to optimize and best qualify the regulatory aspect of these unique and critical requirements. Accordingly a research plan has been submitted, entitled "Controlled Environment Food Processing", which has among its objectives the establishment of the scientific and technological evidence necessary for the production and microbiological control of space food prototypes.

Until this long-term in-house, research program is underway, it is recommended that prompt contractual arrangements be made to evaluate and establish optimal specific analytical methodology for all currrent test microorganisms and products; also to evaluate the significance of other uncommon food-borne pathogens that are not currently included in the requirements, such as those among the clostridia, rickettsia and the enteroviruses. It is further recommended that efforts be made to coordinate with other research groups such as the Air Force School of Aerospace Medicine, Aerospace Systems Division, the development and interpretation of the appropriate clinical evidence related to host susceptibility to autogenous and potentially food-borne infections and intoxication.

B. CURRENT POLICY

This brief report will attempt to reiterate the logic upon which current microbiological requirements have been predicated. An attempt will be made to point out the uniqueness of the space mission; relate the potential effects of flight stress

*El-Bisi, Activities Report, Volume XVII, No. 1, 1965

conditions; identify the primary microbiological objectives; summarize the microbiological evidence related to subject processes and products; suggest a fundamental approach towards effective control of their microbiological quality and safety; and finally, state and justify our current microbiological requirements.

1. Food in The Space Mission

The unique and critical nature of the space mission must be appreciated. Thousands of man-hours and millions of dollars are being spent towards developing fail-safe space vehicles. Similar effort is being aimed toward the proper conditioning and training of the astronauts to assure their effectiveness throughout the space mission.

An essential component of their life support system is food. Food is provided to sustain an appropriate physiological and psychological balance. The microbiological quality of that food is obviously most critical; there must be no doubt regarding its full microbiological safety. It could be an event of incalculable loss if in the middle of a critical space maneuver inside or outside the spacecraft the astronaut suddenly and without warning was struck with an acute case of food-borne gastroenteritis, or possible, fatal neurological intoxication.

2. The Stress Factor

The stress factor must be considered in the course of a space flight. Although there is no direct evidence, it is strongly suspected that inherent and potential stresses in the course of a space flight, such as altitude, cold, heat, weightlessness, and psychological factors, will cause irregularity in the delicate balance between man and microbes. Similar or simulated stresses have been shown in the laboratory to alter the host's resistance to microbial infections and intoxication.

Altitude stress has been reported to render the host more susceptible to virulent strains of Salmonella typhimurium,(1) Klebsiella pneumoniae,(2) and Diplococcus pneumoniae,(3) to gram-negative bacterial endotoxins,(4) and in some cases even after the return of the host to normal atmospheric pressure.(5)

Exposure to sublethal irradiation has been known to diminish host resistance, and even to induce autogenous anaerobic infections from normal microflora in the intestinal tract.(6)

Emotional stress has been reported to enhance viral infection.(7)

Temperature stress both high(8) and low(9) has been shown to cause otherwise avirulent microorganisms to become virulent, including Salmonellae and

staphylococci; to make subminimal doses of salmonella and staphylococcal toxins lethal;[10] and as an influencing factor in making the host more susceptible to enteric viral infections of the Coxsackie types.[11, 12] Even sonic stress can alter susceptibility to viral infection.[13]

The evidence is ample and clear. Environmental and psychological space stresses may diminish the host's resistance to microbial infections; therefore, avirulent microorganisms and levels may become virulent, harmless intestinal saprophytes may provoke autogenous infections, and subminimal toxin levels may provoke overt responses. Until specific clinical evidence is available, all possible measures must thus be taken to eliminate all pathogens, and to minimize the microbial load in all food intake.

3. The Microbiological Objective

It is, therefore, clear that until sufficient and specific evidence is accumulated that would clarify the host's (astronaut's) susceptibility to potential autogenous and exogenous microbial infections and intoxications, effort must be maximized towards keeping his food and environment free from known microbial pathogens or their toxic metabolites throughout his extraterrestrial mission. In this context food will be considered as a potential vector.

a. Food must be produced and packaged under the highest practicable sanitary conditions.

b. The packaged end-products must be free of all known, potentially food-borne microbial pathogens or their toxic metabolites, and allowed to harbor only that minimal load of generally inocuous microbial saprophytes that are usually intrinsic to raw food components and impracticable to remove through the presently utilized technology.

c. The microbiological quality of the packaged end-product must remain unaltered throughout storage, distribution and on-board delivery.

d. On-board practices regarding the preparation, consumption and disposal of food must exclude any condition that would allow microbial propagation or dissemination into the environment.

4. Related Microbiological Evidence

As previously stated no specific evidence has been accumulated on the microbiological aspects of the processing, storage, distribution, preparation and consumption of space food prototypes. It was also stated that current microbiological limits have been based on the best scientific judgment as derived

from already available knowledge and experience. The following is a brief discussion of the major microbiological issues that will have an impact upon the microbiological quality and wholesomeness of the end-product:

a. What inherent microbial types would one expect in the raw materials for the present prototypes?

One would expect a very broad spectrum of the saprophytic and the pathogenic types, since there is a wide range of raw materials - vegetables, fruits, meats, poultry, eggs, fish, cereals, nuts, confection and other components. It must be emphasized here, more than ever before, the need for the highest microbiological quality possible. Prevention is much more effective and much more certain than the cure. This measure becomes particularly more significant when one considers two microbiological phenomena:

(1) A long recognized one, namely, that pre- or in-process microbial growth may leave behind a preformed stable toxin such as staphylococcal enterotoxin.

(2) A recently recognized one, namely, that large dead cell populations of the enteropathogenic gram negative type (such as Salmonellae or Escherichiae) may still be capable of triggering symptomatic pathogenesis.(14, 15) Cellular lipopolysaccharide fractions have been shown to elicit typical gastroenteritis syndrom when administered independently. The cellular preparations are quite thermo-, radio-, and chemo-stable.(16, 17, 18, 19)

b. What lethal or physiological effects would the manufacturing processes have upon such microflora; the primary processes utilized so far being precooking followed by freeze-dehydration?

Precooking is usually terminal and sufficient to destroy all non-sporogenic, pathogenic and saprophytic microflora, except perhaps for a minimal non-pathogenic thermoduric residue, most likely of the streptococci or the micrococci types. Most of the bacterial spore population will survive.

As for freeze-dehydration, the serious lag must be recognized in fundamental microbiological knowledge about conventional (earth-bound) foods, and the total lack of it regarding space food prototypes. It is interesting to note that practically all that is known about the microbiological aspects of freeze dehydration is owed to those engaged in efforts to preserve living cells and cell activities. The little known from the very limited research reports available on foods has given the following broad guide lines:

(1) Either freezing or dehydration will cause a reduction in the nonsporogenic microflora of about 0.1 to 2 log cycles each (12-99%).

(2) Holding in either frozen or dehydrated state may cause an additional slow reduction of about 0.1 to 0.5 log cycles each (12-70%).

(3) Numerous variables influence these lethal effects, such as the type of substrate (food), freezing temperature and rate, holding time and temperature of end item and water activity effects.

(4) The process also exerts some physiological stress on the surviving microflora. The need for specific methodology must, therefore, be emphasized.

c. What health impact might the residual microflora have?

It appears that a well executed, well monitored process should deliver a product practically free of nonsporogenic flora. Goldblith[20] reported that extensive microbiological data from one company on the evaluation of many tons of products, over a three-year period, showed that microbial counts were of very low magnitude on some freeze-dried cooked products and that many lots approached sterility. However, such a product, once reconstituted, must be consumed within the hour or else chilled rapidly to below 40°F or frozen. Sporogenic types (possibly pathogenic) would have a gay time with minimum microbial competition or antagonism, in a reconstituted mishandled product.

d. What potential health hazards may be imparted due to in-plant post-process contaminations?

The main potential health hazard would be imparted through mishandling of the end-product during the final fabrications or packaging through handlers, air, material and equipment. There the health hazard is unlimited.

e. To what degree could such contamination be controlled?

In the manufacturing and assembling of the spacecraft, for certain missions, near asepsis has been required and successfully attained. Such approaches have long been practiced in the manufacturing of pharmaceuticals and biologicals. It is fully justified in this case, and should be economically feasible. The astronaut's health is at least as critical as the hardware he operates.

5. The Fundamental Approach

Having justified the need for a strict microbiological control, identified the microbiological objective and discussed the major related microbiological issues, the following are proposed as the three primary means towards reaching the above objective:

a. Thorough monitoring of the total in-plant operation, from the raw ingredient to the packaged end-product.

b. Institution of sound attainable microbiological requirements for the end-item (Appendix).

c. Institution of appropriate handling code for the end-item by the consumer (NASA).

6. Current Microbiological Requirements

Total Aerobic Plate Count	Not greater than 10,000/gm
Total Coliform Count	Not greater than 10/gm
Fecal Coliform Count	Negative in 1 gm
Fecal Streptococci Count	Not greater than 20/gm
Coagulase Positive Staphylococci	Negative in 5 gm
Salmonellae	Negative in 10 gm

The total aerobic plate count will reflect the overall microbiological quality of the product. Studies by several workers[32, 33, 34, 35] have indicated good correlation between the total count and the incidence of common food-borne pathogens. The methodology selected for determining the total count (Appendix) has been recommended by several authoritative sources.[26, 27, 36]

The total coliform count has been utilized as a general indicator of fecal pollution in food, milk and water for many years.[26, 27, 30, 37] Because of the ubiquity of the wild coliform types, this estimate does not necessarily reflect fecal pollution. Their presence, however, will cause suspicion and make further testing mandatory. Their presence in relatively high numbers will also indicate poor in-process control.

Foods containing coliforms are further tested for the presence of the Escherichia coli type. This organism is a common inhabitant of the intestinal tract of man and warm-blooded animals; its presence will indicate the potential presence of enteric pathogens. Although generally regarded as harmless, the pathogenicity of certain strains of E. coli has been reported.[38, 39, 40] The methods selected for the enumeration of coliforms and E. coli type have been again recommended by several authoritative sources.[26, 27, 28, 29, 36, 37]

The fecal streptococci were first associated with food borne gastroenteritis outbreaks in 1926[41] and outbreaks of streptococcal food poisoning have been reported by several investigators since then.[45, 46, 47, 48] Experimental enterococcal infections have also been established in human volunteers by some workers.[42, 45, 46, 49] In addition a great deal of circumstantial evidence has been accumulated over the years where the streptococci represented the predominant

flora in foods implicated in food poisoning outbreaks. The methodology selected for the enumeration of the fecal streptococci has been again recommended by several authoritative sources.[27, 31]

Staphylococcal food poisoning is caused by the enterotoxins of Staphylococcus aureus. The pathogenicity of this organism and its role as the etiologic agent in food poisoning outbreaks is well established.[42, 44] It has been reported that in 1968 the staphylococcal food poisoning was the most commonly reported type and accounted for nearly 25% of all outbreaks and 25% of the cases.[43] The method selected has been again recommended by several authoritative sources.[26, 27, 36, 50]

Salmonellosis is probably the most common or well known type of food-borne illness and has been recognized since 1856. Salmonella outbreaks in the United States have increased from 39 in 1967 to 42 in 1968 and involved over 1200 patients.[43] Although Salmonella typhimurium is the principal serotype connected with human salmonellosis any of the other 1000 recognized serotypes are potentially capable of causing a clinical infection. Again, the method selected has been supported by several authoritative sources.[26, 27, 36, 51, 52]

It should be noted that the above stated microbial indices have been selected because they are commonly used as indicators or potential food-borne pathogens. Other indices are not included due to inadequate technological, epidemiological and clinical evidence or well established analytical methodology.

The tolerance levels specified for the total coliform and the streptococcal counts are typical of sound technology and are readily attainable.

In case of E. coli, staphylococcus and salmonella the food mass to be shown as negative for the contaminant is set according to the sensitivity limit inherent in the method used.

It may be stated that adherence to the above microbial requirements and maintaining a strict in-plant, in-process and on-board sanitary practice will assure maximal attainable, although not absolute, safety. This has been supported by accumulated production experience (Tables 1[a,b,c,d] and 2).

It is urged, however, that process and product specific studies be conducted to elicit clearly the etiological significance of these organisms, organisms such as Clostridium perfringens and others that are less known, including viruses, rickettsia, vibrios, mycoplasma.

It should be further noted that the methodology has been carefully selected for efficiency and reliability. Although experience has proved the current methods to be satisfactory, the prompt initiation of a specific comparative

study is urged to ascertain which methods are most optimal for these test products.

There is an awareness, however, of the infinite controversy that plagues this subject, be it the microbial types, limits or methods. This situation has been studied carefully and what is recommended is that which is considered to be the best scientific judgement in the absence of specific laboratory and clinical evidence. However, constructive criticism and cooperation from interested agencies working in this area is welcomed.

C. FUTURE PLANS

1. Initiate in-house fundamental and applied studies on the microbiological aspects of space food processes and products. These studies should establish the specific scientific and technological evidence, upon which regulatory aspects of this unique procurement can be based.

2. Initiate immediate contractual studies to:

a. Establish specific optimal analytical methodology for current space food prototypes.

b. Evaluate the significance of clostridia, rickettsia, vibrios and other uncommon food-borne bacterial pathogens in the production and control of space food prototypes.

3. Continue and expand investigations related to the significance of viruses in the production and control of space food prototypes.

TABLE Ia. MICROBIOLOGICAL ANALYSIS OF RANDOM SPACE FOOD SAMPLES DURING 1967 - 1968

MEATS (PRECOOKED, DEHYDRATED)

Samples	Total Counts per gram	Total Coliform per gram	Fecal Coliform per gram	Fecal Strep. per gram	Coag.Pos. Staph.per 5 gram	Salmon. per 5 gram
RD 1522						
Beef BBQ Bites	2,500	<10	Neg	<10	Neg	Neg
#146						
Beef BBQ Bites	2,100	<10	Neg	<10	Neg	Neg
RD 1634						
Beef Hash	3120	<10	Neg	<10	Neg	Neg
RD 1635						
Chili con carne.....	<100	<10	Neg	<10	Neg	Neg
Beef patties ...	6500	<10	Neg	<10	Neg	Neg
RD 1521						
Beef Hash Bars	5000	14	Neg	<10	Neg	Neg
RD 1636						
Spaghetti and Meat Balls.....	12,100	<10	Neg	<10	Neg	Neg
RD 1637						
Beef with Rice	9,740	<10	Neg	19	Neg	Neg
RD 1638						
Chicken Stew....	3,680	<10	Neg	<10	Neg	Neg
RD 1639						
Pork with Esc potatoes ...	1,080	<10	Neg	<10	Neg	Neg
RD 1565						
Turkey Bites ..	<100	<10	Neg	<10	Neg	Neg
RD 1566						
Turkey Bites..	<100	<10	Neg	<10	Neg	Neg
Turkey w/Gravy	<100	<10	Neg	<10	Neg	Neg
RD 1640						
Beef Stew	4,820	<10	Neg	<10	Neg	Neg
RD 1641						
Chicken & Rice..	1,820	<10	Neg	<10	Neg	Neg
#500						
Beef Stew.....	940	<10	Neg	<10	Neg	Neg

TABLE Ib. MICROBIOLOGICAL ANALYSIS OF RANDOM SPACE FOOD SAMPLES DURING 1967 - 1968

CANDY AND NUTS (DEHYDRATED)

Samples	Total Count per gram	Total Coliform per gram	Fecal Coliform per gram	Fecal Strep. per gram	Coag.Pos. Staph.per 5 gram	Salmon. per 5 gram
RD 1528 Chocolate Cubes	26,000	<10	Neg	<10	Neg	Neg
95-16 Chocolate Cubes	32,100	<10	Neg	<10	Neg	Neg
RD 1410 Chocolate Cubes	60,600	<10	Neg	<10	Neg	Neg
RD 1407 Chocolate Oatmeal Bar	12,300	<10	Neg	<10	Neg	Neg
RD 1529 Peanut Cubes....	820	<10	Neg	40	Neg	Neg
RD 1434 Peanut Cubes....	370	<10	Neg	108	Neg	Neg
RD 914 Peanut Cubes....	795	<10	Neg	--	Neg	Neg
RD 921 Peanut Cubes....	675	<10	Neg	<10	Neg	Neg
RD 920 Peanut Cubes....	550	<10	Neg	<10	Neg	Neg
RD 1406 Cherry Nut Bar..	120	<10	Neg	<10	Neg	Neg
RD 1408 Date Nut Bar....	2,400	<10	Neg	<10	Neg	Neg
RD 916 Coconut Cubes...	405	<10	Neg	--	Neg	Neg
RD 948 Coconut Cubes...	1,000	<10	Neg	75	Neg	Neg

TABLE Ic. MICROBIOLOGICAL ANALYSIS OF RANDOM SPACE FOOD SAMPLES DURING 1967 - 1968

BEVERAGES (DEHYDRATED)

Samples	Total Count per gram	Total Coliform per gram	Fecal Coliform per gram	Fecal Strep. per gram	Coag.Pos. Staph.per 5 gram	Salmon. per 5 gram
RD 1516 Orange Drink..	<100	<10	Neg	<10	Neg	Neg
RD 1561 Orange Drink..	<100	<10	Neg	<10	Neg	Neg
RD 1119 Orange Drink...	180	<10	Neg	<10	Neg	Neg
RD 1416 Orange Drink with Dextrose...	40	<10	Neg	<10	Neg	Neg
RD 1431 Orange-Grapefruit with Dextrose	40	<10	Neg	<10	Neg	Neg
RD 1433 Grapefruit Drink with Dextrose & Sucrose	180	<10	Neg	<10	Neg	Neg
RD 1572 Grapefruit Drink	160	<10	Neg	<10	Neg	Neg
RD 1573 Orange-Grapefruit	400	<10	Neg	<10	Neg	Neg
RD 1574 Orange-Pineapple	40	<10	Neg	<10	Neg	Neg
RD 1584 Grapefruit Drink	200	<10	Neg	<10	Neg	Neg
RD 1586 Orange-Pineapple	300	<10	Neg	<10	Neg	Neg
14 Assorted Fruit Drinks..	<100	<10	Neg	<10	Neg	Neg
RD 1489 Chocolate Metrecal.....	1700	<10	Neg	<10	Neg	Neg

TABLE Ic. MICROBIOLOGICAL ANALYSIS OF RANDOM SPACE FOOD SAMPLES DURING 1967 - 1968 (Cont'd)

BEVERAGES

Samples	Total Count per gram	Total Coliform per gram	Fecal Coliform per gram	Fecal Strep. per gram	Coag.Pos. Staph.per per 5 gram	Salmon. per 5 gram
RD 1499 Chocolate Metrecal.....	2000	<10	Neg	82	Neg	Neg
RD 1414 Chocolate Metrecal	1500	<10	Neg	260	Neg	Neg
RD 1490 Vanilla Metrecal	1700	<10	Neg	170	Neg	Neg
RD 1498 Vanilla Metrecal	1900	16	Neg	279	Neg	Neg
RD 1413 Vanilla Metrecal	2500	127	Neg	23	Neg	Neg
RD 1495 Black Coffee(2)	<100	<10	Neg	<10	Neg	Neg
RD 1563 Coffee w/cream	<100	<10	Neg	<10	Neg	Neg
RD 1496 Coffee w/sugar	>300,000	<10	Neg	<10	Neg	Neg

TABLE Id. MICROBIOLOGICAL ANALYSIS OF RANDOM SPACE FOOD SAMPLES DURING 1967 - 1968

MISCELLANEOUS (DEHYDRATED)

Samples	Total Count per gram	Total Coliform per gram	Fecal Coliform per gram	Fecal Strep. per gram	Coag.Pos. Staph.per 5 gram	Salmon. per 5 gram
RD 1537 Instant Apple-sauce	40	< 10	Neg	< 10	Neg	Neg
RD 1538 Instant Apple-sauce	20	< 10	Neg	< 10	Neg	Neg
RD 1541 Freeze Dried Strawberries	720	< 10	Neg	< 10	Neg	Neg
RD 1593 Chicken Soup and Gravy	57,000	2	Pos	2650	Neg	Pos
RD 1594 Beef Soup and Gravy	17,800	< 10	Neg	33	Neg	Pos
FFC 67 Fruit Cock-tail	230	< 10	Neg	< 10	Neg	Neg
FFC 19 Fruit Cock-tail	150	< 10	Neg	< 10	Neg	Neg
RD 1527 Vanilla Ice Cream Cubes	160	< 10	Neg	< 10	Neg	Neg
RD 1523 Chocolate Ice Cream Cubes	20,000	< 10	Neg	< 10	Neg	Neg
RD 1551 Chili & Beans	1,600	< 10	Neg	< 10	Neg	Neg
RD 940 Creamed Corn Powder	5,650	< 10	Neg	< 10	Neg	Neg

TABLE 2. SUMMARY OF MICROBIOLOGICAL ANALYSIS
OF RANDOM SPACE FOOD DURING 1967 - 1968

	No. of Samples	Percent Samples Containing (per gram)								
		<10	<20	<50	<100	<1000	<10,000	<50,000	<100,000	>100,000
Total Plate Count	75			7	37	61	88	96	99	1
Total Coliform Count	77	96	99	99	99	100				
Fecal Streptococci Count	73	85	86	90	93	100				

		Percent Samples Positive	Percent Samples Negative
Fecal Coliforms	75	1.3	98.7
Coagulase Positive Staphylocci	75	0	100
Salmonellae	75	3	97

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APPENDIX. Current Microbiological Requirements and Respective Methodology

Space Food Prototype
Production Guide
Addendum No. 1E
U.S. Army Natick Labs.
Natick, Massachusetts
27 March 1969
Superseding Addendum No. 1D
1 December 1967

MICROBIOLOGICAL REQUIREMENTS FOR SPACE FOOD PROTOTYPES

I. Microbiological Requirements

Total Aerobic Plate Count	Not greater than	10,000/gm
Total Coliform Count	Not greater than	10/gm
Fecal Coliform Count	Negative in	1 gm
Fecal Streptococci Count	Not greater than	20/gm
Coagulase Positive Staphylococci	Negative in	5 gm
Salmonellae	Negative in	10 gm

II. Methodology

A. Preparation of slurry:

1. Twenty-five (25) grams of the dehydrated sample are aseptically transferred to a sterile blender cup. Add 225 ml of chilled sterile buffered water (SBW: PO_4 M/15, pH 7.0)[2] and blend for two minutes. This slurry constitutes a 1:10 dilution and contains the equivalent of 0.1 gm food sample per ml. Hereafter this dilution shall be termed Extract A.

2. Extracts shall be maintained at no greater than 5°C until promptly used as prescribed in the following tests.

B. Total Aerobic Plate Count:

1. Transfer ten (10) -ml of Extract A into a 90-ml SBW, giving a final dilution of 1:100.

2. Transfer one (1)-ml of the 1:100 dilution into each of five (5) Petri-plates and pour with "Plate Count" agar (Difco).(1, 3)

3. Incubate plates at 35°C and count after 48 hours.

4. The total number of colonies on the five (5) plates shall not exceed 500.

C. Total Coliform Count:

1. Transfer one (1)-ml of Extract A into each of ten (10) Petri-plates and pour with "Violet Red Bile" agar (VRB).(1, 3)

2. Stratify solidified plates with 5 ml of VRB agar..

3. Incubate plates at 35°C and count typical coliform colonies (dark red, 0.5 mm or more in diameter) after 18 to 24 hours.

4. The total number of typical colonies on all ten (10) plates shall constitute the "Total Coliform Count" per 1.0 gm of food and shall not exceed ten (10).

In case the "Total Coliform Count" exceeds ten (10), the product is deemed unacceptable, and further testing is, therefore unwarranted.

D. Fecal Coliform Count:

1. Transfer each typical VRB colony (see C.3) into phenol red lactose broth fermentation tubes.

2. Incubate at 35°C for 18-24 hours.

3. Transfer two loopfulls (3-mm diameter) of broth from each positive tube (displaying acid and gas) into an "EC" broth fermentation tube.(3, 4, 5)

4. Incubate at 45.5 ± 0.2°C for 24 hours. Both temperature and time are critical for this differential test. Hence, incubation shall be carried out in a constant-temperature bath, monitored with a certified Bureau of Standards thermometer or equivalent. Incubation time shall not exceed 24 hours. "EC" tubes displaying gas production are considered positive for "Fecal Coliforms". A single "EC-positive" culture shall constitute rejection.

5. Where merited, the analyst may further confirm the EC-positive cultures for the Escherichia coli type through the establishment of their IMVic pattern according to Standard Procedures.

E. Fecal Streptocci Count

1. Transfer one (1)-ml of Extract A into each of ten (10) Petriplates and pour with "KF Streptococcus" Agar.(3, 5)

2. Incubate plates at 35° C for 48 hours and count all red or pink colonies.

3. The total number of typical colonies of all ten "KF" plates shall constitute the "Fecal Streptococci Count" per 1.0 gm of food and shall not exceed twenty (20).

4. Where merited, the analyst may further confirm the KF-positive colonies through:

a. Microscopic examination

b. Gram stain

c. Catalase test

d. Growth in Ethyl Violet Azide (EVA) broth.

F. Coagulase Positive Staphylococci:

1. Transfer 50-ml of Extract A into 200 ml of cooked meat medium with NaCl[3]. The medium is prepared overstrength by adding 31.25 gm of cooked meat medium and 24.5 gm of NaCl to 200 ml of distilled water. The final concentration in 250 ml will be 12.5% and 10% respectively.

2. Incubate at 35°C for 24 hours.

3. Spread one-tenth (1/10)-ml of cooked meat medium on each of two (2) plates of Vogel and Johnson (VJ) agar [8].

4. Incubate VJ plates at 35°C and examine after 24 and 48 hours for the presence of black colonies with yellow zones.

5. From each plate transfer two or more representative colonies which have reduced tellurite to brain heart infusion (BHI) tubes, and incubate at 35°C for 24 hours.

6. Remove the remainder of the colony with a loop and emulsify in 0.2 ml of BHI. Add 0.5 ml of coagulase plasma, mix and incubate in a 35°C water bath for 4 hours (if time does not permit, use the 24 hour culture).

7. Note those tubes which are negative and repeat the coagulase test with the 24 hour culture.

8. A single coagulase positive colony shall constitute rejection.

G. Salmonellae:

1. Transfer 100 ml of Extract A into 100 ml of double strength lactose broth.

2. Incubate at 35°C for 24 hours.

3. Transfer 25 ml of lactose broth into 225 ml of each of Selenite-Cystine broth and TT broth base (modified TETRATHIONATE BROTH containing brilliant green (1:100,000) [3,9,11].

4. Incubate at 35°C for 18-24 hours.

5. From each enrichment culture streak a loopful on one plate each of three (3) selective media: Brilliant Green Sulfadiazine (BGS) agar, Bismuth Sulfite (BS) agar, and Salmonellae-Shigellae (SS) agar.

6. BGS and SS plates are incubated for 24 hours and BS plates for 48 hours at 35°C. Typical colonies are pink to deep fuschia on BGS, black on BS, smooth and colorless on SS.

7. Pick with a needle two typical colonies from each plate having growth. Inoculate "Triple Sugar Iron" agar (TSI) slant by stabbing the butt and streaking the slant and streak on "Christensons Urea" agar (CU) slant.

8. Incubate all slants at 35°C for 24 hours.

9. Observe CU slants periodically every 4 to 6 hours. If culture shows an urea-positive reaction (reddening of agar) the respective colony is Salmonellae negative and test is ended.

10. Positive TSI (acid butt, alkaline slant, with and without gas and H_2S) associated with urea-negative reaction shall constitute a presumptively positive Salmonellae culture.

11. Transfers from positive TSI slants are typed against salmonellae O and H polyvalent antisera. Positive reaction constitutes confirmed presumptively positive salmonellae in the test sample.

12. Presumptively positive TSI cultures are further confirmed through reactions in the following:

a. Dulcitol (+)[11], malonate (-) broths [11].

b. Lysine decarboxylase broth (+).

c. KCN broth (-).

d. Indole broth (-).

13. A single confirmed positive Salmonellae culture shall constitute rejection.

H. General Provision:

1. Due to the special nature of certain dehydrated menu components, the prescribed dilution schedule may be modified in order to facilitate appropriate pipetting of the test material. Higher dilutions may be prepared and used for subculturing, providing the total equivalent mass of the subcultured test component remains the same as currently prescribed.

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13. ABSTRACT

The microbiological requirements for space foods were established in 1964 to safeguard the health of the astronauts during a space mission. These requirements are: the total aerobic plate count shall not exceed 10,000 per gram; the total coliform count shall not exceed 10 per gram; the fecal coliforms shall be negative in one gram; the fecal streptococci shall not exceed 20 per gram; the coagulase positive staphylococci shall be negative in five grams; and the Salmonellae shall be negative in ten grams of food.

During 1967 and 1968, 88 per cent of the space foods tested had total plate counts of less than 10,000 per gram; 96 percent had less than 10 coliforms per gram and 99 percent were negative for fecal coliforms; 86 percent has less than 20 streptococci per gram; and 100 percent were negative for staphylococci and Salmonellae.

This report discusses the scientific and technical rationale behind these microbiological requirements. These requirements and the methodology prescribed for determining compliance therewith are documented. Both are under continued review and amendment, in keeping with up-to-date scientific knowledge and technical experience.

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